

AMENDMENTS TO CLAIMS:

The following listing of claims will replace all prior versions and listings of claims in the application.

Claim Listing:

1-21. (Canceled)

22. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

P_x-S_x-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

P_x is a yeast ADH2 promoter sequence;

S_x is a nucleic acid encoding ~~a signal sequence or~~ an α factor leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons when n=1-15, respectively;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, and As_m is 1-10 codons when m=1-10, respectively;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

isolating said fusion protein from said fermentation supernatant,

wherein said isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

23. (Currently Amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising:

(A) culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

$P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T$

where

P_x is a yeast ADH2 promoter sequence;

S_x is a nucleic acid encoding ~~a signal sequence or~~ an α factor leader sequence;

B_n is a chemical bond or a codon, wherein $n=0-15$; B_n being a chemical bond when $n=0$ and B_n being 1-15 codons when $n=1-15$, respectively;

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein $m=0-10$; As_m is a chemical bond when $m=0$, and As_m is 1-10 codons when $m=1-10$, respectively;

R in $As_m R$ is an arginine codon or a chemical bond;

$protein(Y)$ is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

(B) separating the fermentation supernatant from the host cell;

(C) culturing the host cell in fresh medium;

(D) separating the resulting supernatant from the host cell;

(E) repeating (B) and (C) and (D) several times; and

(F) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

24. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a yeast ADH2 promoter sequence;

Sx is a nucleic acid encoding ~~a signal sequence or~~ an α factor leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons when n=1-15, respectively;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, and As_m is 1-10 codons when m=1-10, respectively;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

isolating said fusion protein from said fermentation supernatant,

wherein the isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising releasing the protein encoded by protein(Y)

from the fusion protein and concentrating said protein encoded by protein(Y) by microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

25-29. (Canceled)

30. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T

where

P_x is a yeast ADH2 promoter sequence;

S_x is a nucleic acid encoding ~~a signal sequence or~~ an α factor leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons when n=1-15, respectively;

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, and As_m is 1-10 codons when m=1-10, respectively;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell;

isolating said fusion protein from said fermentation supernatant, and

releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

31. (Currently Amended) A nucleic acid sequence comprising:

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a yeast ADH2 promoter sequence;

Sx is a nucleic acid encoding ~~a signal sequence or~~ an α factor leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15 and B_n is a chemical bond when n=0 or B_n is 1-15 codons[[,]] when n=1-15, respectively ~~is an integer from 1 to 15, or a chemical bond, when n=0;~~

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

(As_mR) is an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg);

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence.

32. (Previously Presented) The nucleic acid sequence of claim 31 in which (As_mR) encodes SEQ ID NO: 12 (Gly-Asn-Ser-Ala-Arg).

33-34. (Canceled)

35. (Previously Presented) The nucleic acid of Claim 31 wherein Hir is a nucleic acid sequence encoding lepirudin.

36. (New) The process according to any one of claims 22-24 or 30, wherein said host cell is a yeast cell.

37. (New) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

$P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T$

where

P_x is a promoter sequence;

S_x is a nucleic acid encoding a signal sequence or leader sequence;

B_n is a chemical bond or a codon, wherein $n=0-15$; B_n being a chemical bond when $n=0$ and B_n being 1-15 codons when $n=1-15$, respectively;

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein $m=0-10$; As_m is a chemical bond when $m=0$, and As_m is 1-10 codons when $m=1-10$, respectively;

$protein(Y)$ is a nucleic acid sequence encoding mini-proinsulin; R in $As_m R$ is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell is a yeast cell and comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

isolating said fusion protein from said fermentation supernatant,

wherein said isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

38. (New) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising:

(A) culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

$P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T$

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons when n=1-15, respectively;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, and As_m is 1-10 codons when m=1-10, respectively;

R in As_m R is an arginine codon or a chemical bond;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell is a yeast cell and comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

(B) separating the fermentation supernatant from the host cell;

(C) culturing the host cell in fresh medium;

(D) separating the resulting supernatant from the host cell;

(E) repeating (C) and (D) several times; and

(F) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

39. (New) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons when n=1-15, respectively;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, and As_m is 1-10 codons when m=1-10, respectively;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell is a yeast cell and comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell and

isolating said fusion protein from said fermentation supernatant,

wherein the isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising releasing the protein encoded by protein(Y) from the fusion protein and concentrating said protein encoded by protein(Y) by microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

40. (New) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

B_n is a chemical bond or a codon, wherein $n=0-15$; B_n being a chemical bond when $n=0$ and B_n being 1-15 codons when $n=1-15$, respectively;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

As_m is a chemical bond or codon, wherein $m=0-10$; As_m is a chemical bond when $m=0$, and As_m is 1-10 codons when $m=1-10$, respectively;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell is a yeast cell and comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell;

isolating said fusion protein from said fermentation supernatant, and

releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

41. (New) The process according to any one of claims 37-40, wherein said Px is a yeast ADH2 promoter, and Sx is an α factor leader sequence.